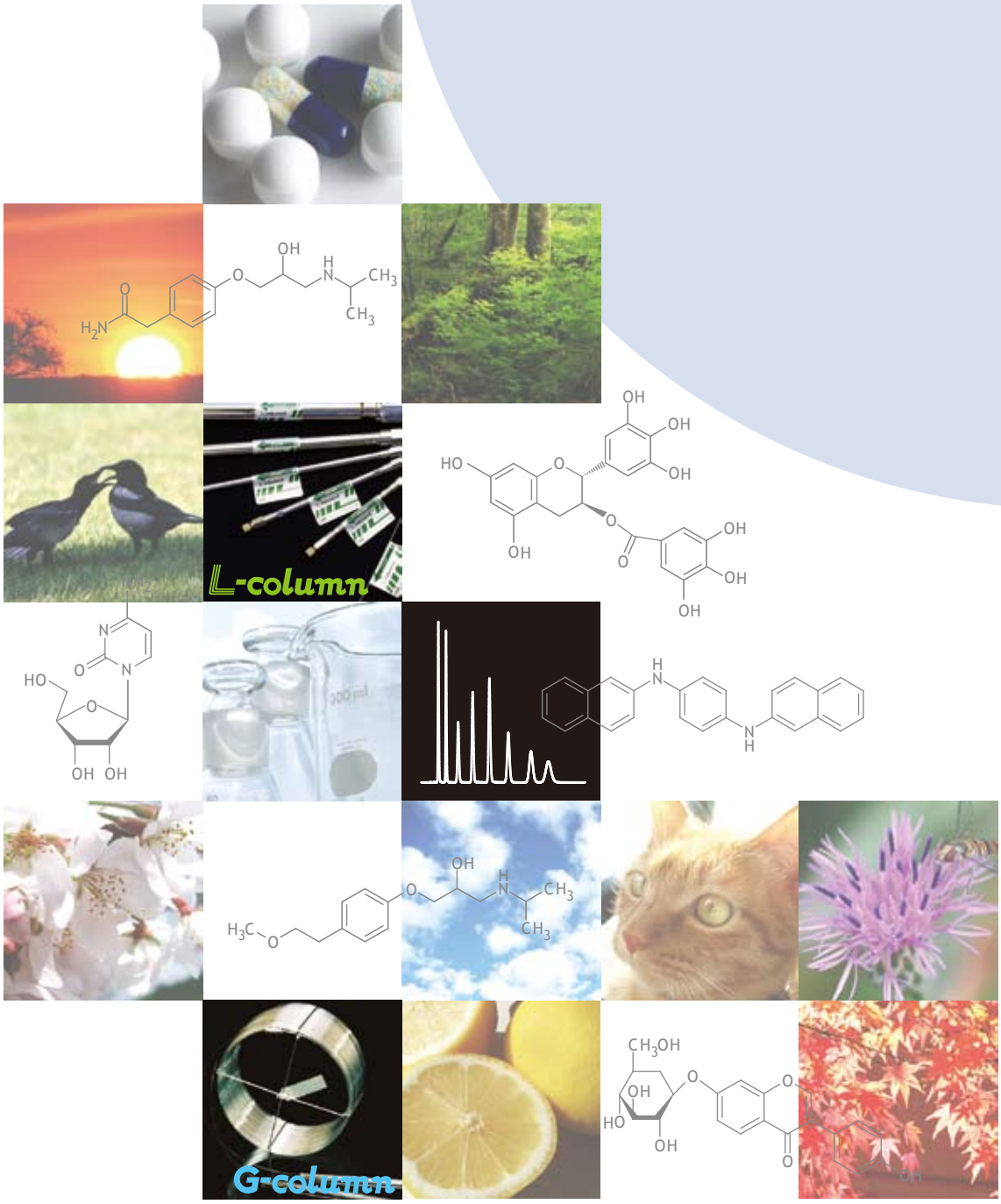
Ver.06

Column catalog

L-column

High performance columns for LC



CERI

Introduction

CERI serves in creating harmony between human beings, chemicals, and environments.

Chemicals Evaluation and Research Institute, Japan (CERI) engages in a wide scope of activities related to chemicals, including testing, analysis, evaluations, and research and development. Our ultimate objective is to promote the sound development of industry and enhance people's lifestyles by upgrading the quality of chemicals and assuring their safety, while at the same time emphasizing environmental preservation and the protection of health.
As an independent, unbiased organization, CERI plays a supporting role in the research and development projects of clients utilizing its services. Working in state-of-the-art facilities, our highly qualified professionals conduct tests, analysis, research, and studies in the field of chemicals.
CERI carries out basic research, development of testing methods, development of techniques to assess risk, data collection and analysis, and other related functions in terms of the entire life cycle of chemicals from their development to their production, consumption and abandonment. As an institute that performs such comprehensive evaluations, we will continue to count on the ongoing support and cooperation of our clients in the world of chemicals.

Chromatography plays an important role in the analysis field. CERI has been developing convenient and high performance columns based upon our experiences as users of chromatographs since 1984. During this time we have developed and supplied products for HPLC and GC including ***G-column*** for gas chromatography, wide-bore, open tubular columns, and ***L-column*** and ***L-column2*** for high performance liquid chromatography. We respond rapidly to new technology and help your test, research and development.

Quality control

Our quality management system is assessed and verified to be in accordance with ISO 9001: 2008 for the design, development, production and supply of columns for gas chromatography and high performance liquid chromatography (Fig. 1).
We maintain probing inspection systems of raw materials and production processes at each step. All finished products are thoroughly inspected. These steps ensure a tight control of quality.We are continuously refining our operations to supply products for the satisfaction of our customers.

Fig. 1 Certificate of registration.

L-column series

Column developed in pursuit of convenience and high performance

L-column series is the column for reversed-phase high-performance liquid chromatography (RP-HPLC) that enabled separation only by hydrophobic interaction because of inhibition of the secondary interaction with residual silanol groups and metal impurities. In 1990, the new end-capping method using high-temperature silylation was invented. The performance of ***L-column ODS*** developed using this method was far superior to that of existing C18 columns, and it became a pioneer of the new-generation end-capping method. ***L-column2 ODS***, surpassing ***L-column ODS*** in performance, was developed by upgrading the end-capping method. There is now a demand for RP-HPLC columns capable of both handling various issues of trace analysis due to the popularization of LC/MS(/MS) and providing advanced analytical precision. ***L-column2 ODS*** is an ideal column for meeting this demand.

■ *L-column* Lineup

	Particle size (μm)	Pore size (Å)	Surface area (m^2/g)	Carbon conten(%)	Bonded phaseC18	USP category
L-column2 ODS	2, 3, 5	120	340	17	C8	L 1
L-column2 C8	5	120	340	10	C18	L 7
L-column ODS	3, 5	120	340	17	C18	L 1
L-column ODS-P	5	300	150	9	C18	L 1

Column size

We have columns with inner diameters for a wide range of uses from the nano column with a 0.075-mm inner diameter to the preparative column with a 50-mm inner diameter.

L-column2 ODS

Next generation high performance silica-based ODS column

Average particle size	2 µm, 3 µm, 5 µm
Average pore size	120 Å
Range of pH	pH 2–9
USP category	L1

L-column2 ODS exceeds even the high performance of ***L-column ODS*** by virtue of its advanced new end-capping method. It accommodates the analysis of acidic, basic and chelating compounds.

■ Characteristics of *L-column2 ODS*

- Sharper peaks for acidic, basic and chelating compounds due to extremely low silanol adsorption.
- Superior peak shapes in both acetonitrile/water and methanol/water mobile phases makes ***L-column2 ODS*** convenient to use.
- Economical due to high durability in a wide range of pH and temperature.
- Uniform lot to lot reproducibility of analyses due to extensive quality control measures.

■ Residual silanol groups

The level of residual silanol groups is measured by FT-IR spectrum. The spectra of C18 without end-capping and the fully end-capped ***L-column2 ODS*** are shown (Fig. 2). The spectrum region for C-H and O-H provides quantitative information as well as qualitative identification. FT-IR spectra show virtually no presence of silanol groups on ***L-column2 ODS***. In addition, the spectrum region for O-H (the right spectra) shows that ***L-column2 ODS*** has the least residual silanol groups of any column tested.

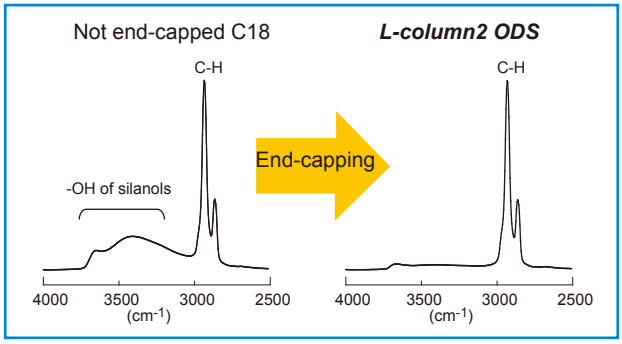


Fig. 2 FT-IR spectra for ***L-column2 ODS***.

■ Comparison between *L-column2 ODS* and other columns

Basic compounds show peak tailing due to their adsorption by residual silanol groups. The shape of the peaks depends notably on the amount of silanol groups (Fig. 3).

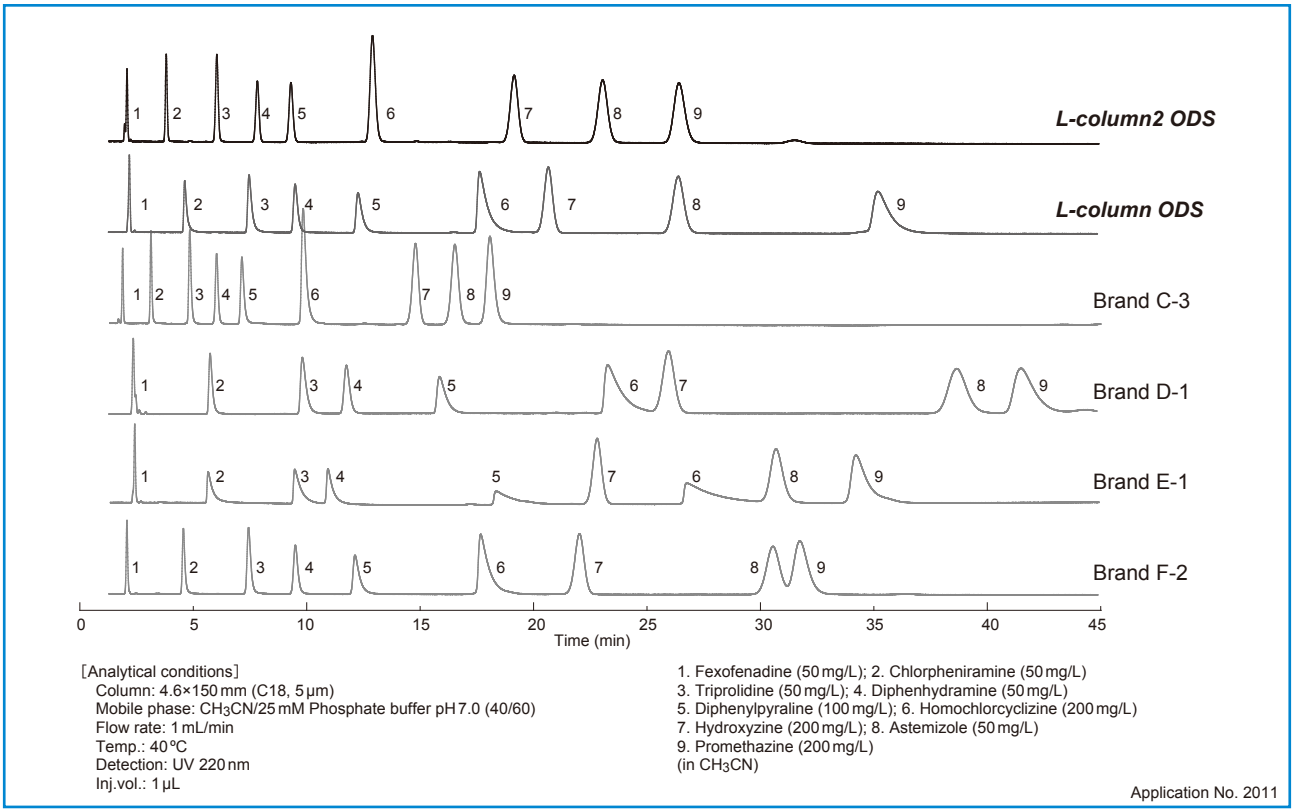


Fig. 3 Chromatograms of basic compounds using ***L-column2 ODS*** and other columns.

Acidic compounds also show peak tailing on poorly end-capped columns. Superior-performance columns provide sharp peaks of basic and acidic compounds.

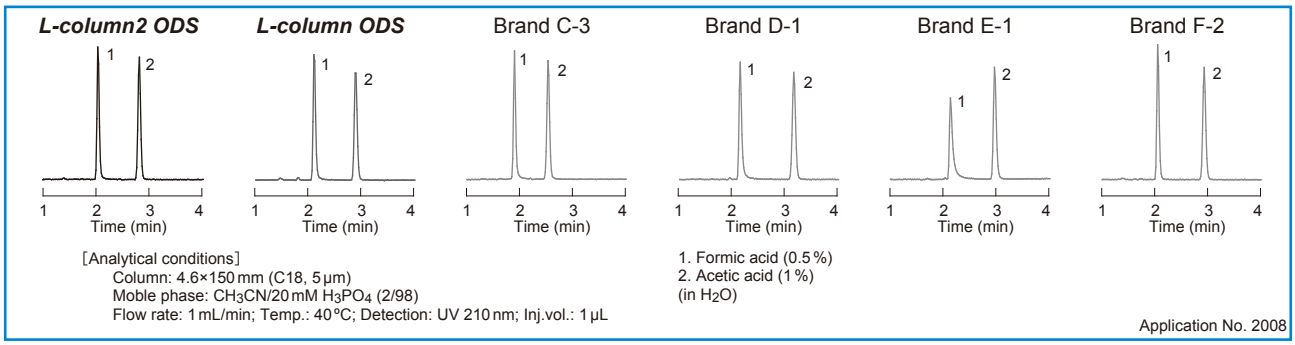


Fig. 4 Chromatograms of acidic compounds, formic acid and acetic acid, using ***L-column2 ODS*** and other columns.

Chelating compounds are adsorbed by metal impurities present on the surface of base silica gels. Fewer metal impurities and higher end-capping surface coverage provide sharper peaks of chelating compounds.

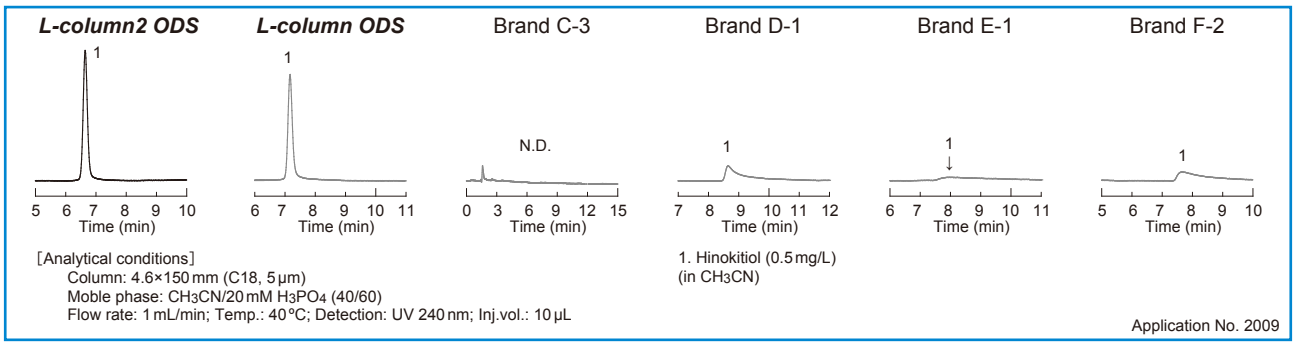


Fig. 5 Chromatograms of chelating compound, hinokitiol, using ***L-column2 ODS*** and other columns.

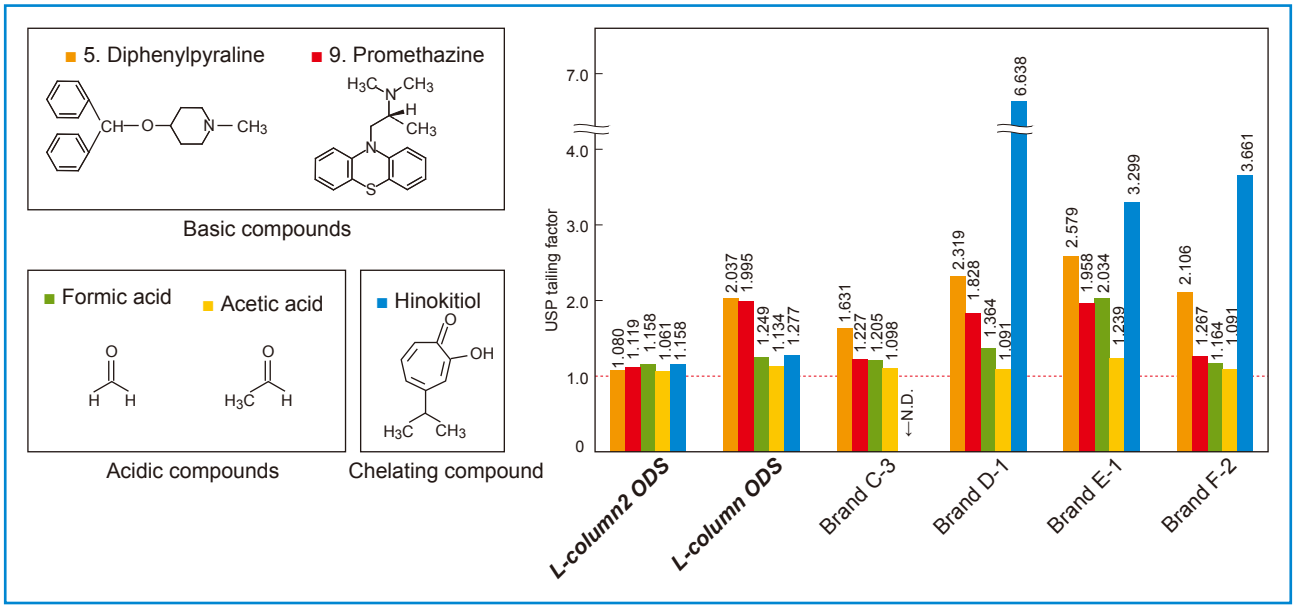


Fig. 6 Comparison between tailing factors of the adsorptive compounds for ***L-column2 ODS*** and those for other columns.

■ Low adsorption

Basic compounds show peak tailing due to their adsorption by residual silanol groups. Therefore, adsorption is inhibited using an acidic mobile phase or a mobile phase including methanol to control peak tailing. Because the residual silanol groups show intrinsic activity using a neutral mobile phase or a mobile phase including acetonitrile as an organic solvent, peak tailing of basic compounds due to adsorption occurs when using poorly end-capped columns.

L-column2 ODS is perfectly end-capped, so it does not adsorb analytes using any composition of mobile phases and provides a superior peak shape. While peak tailing of basic compounds using a neutral mobile phase or a mobile phase including acetonitrile occurs with almost all C18 columns, peak tailing of basic compounds using these mobile phases does not occur with ***L-column2 ODS*** (Fig. 7). Therefore, it can be used in a wide range of compositions of mobile phases. This is an important point when selecting a column.

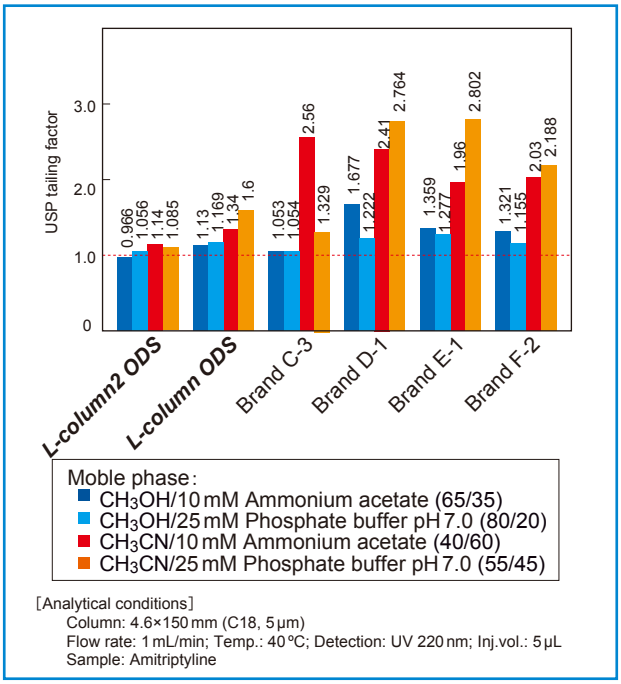


Fig. 7 Difference of tailing factor by kind of solvent (amytriptyline).

■ Improved durability

A durability test was carried out under high temperature conditions which accelerate deterioration of columns. ***L-column2 ODS*** was stable for the longest time. Although it is silica-based, it shows superior durability even under alkaline conditions due to the extremely dense end-capping.

[Accelerated acidic mobile phase lifetime test]
Under acidic conditions, below pH 1, both the end-capping group and the ODS group are hydrolyzed. Retention time decreases with the decrease of ODS groups. Resolution decreases with the progression of the hydrolysis. ***L-column2 ODS*** resists hydrolysis even under these harsh conditions to maintain retention and resolution for an extended lifetime (Fig. 8).

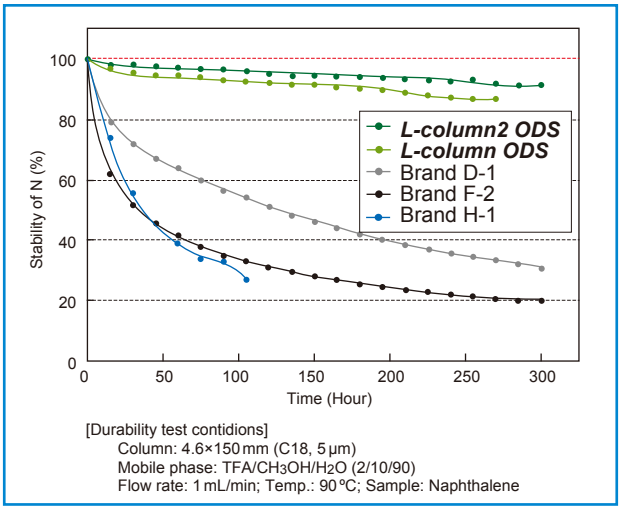


Fig.8 Accelerated durability test with acidic mobile phase (under pH 1).

[Accelerated alkaline mobile phase lifetime test]
Dissolution of the base silica is accelerated in alkaline mobile phase. Efficiency (theoretical plate number) drops suddenly in these conditions. ***L-column2 ODS*** has superior durability under high pH conditions (pH 10) due to the protection of the silica surface afforded by the advanced end-capping process (Fig. 9).

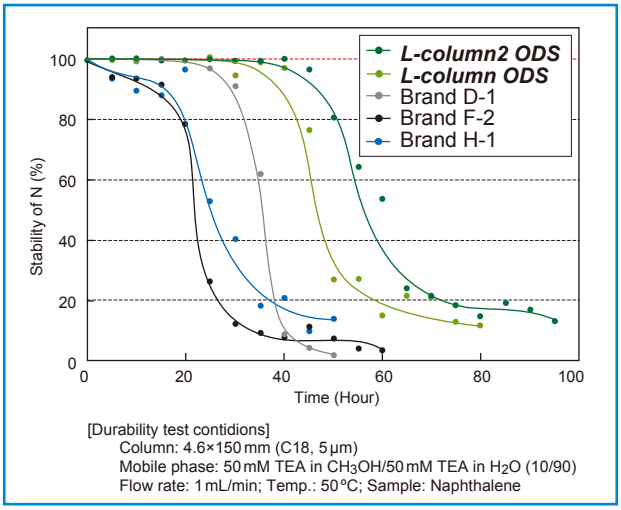
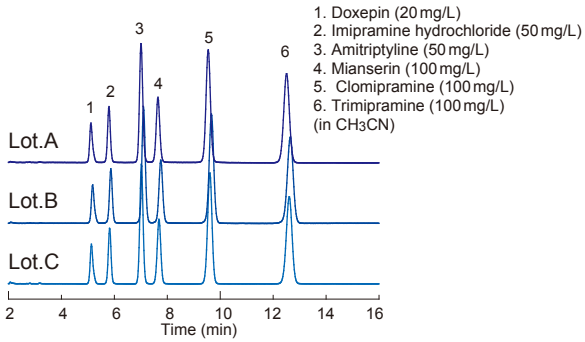


Fig.9 Accelerated durability test with alkaline mobile phase (pH 10).

■ Superior reproducibility

Variation between product lots due to residual silanol groups is prevented by superior end-capping. Although reproducibility of retention times of basic compounds is poor between product lots, the coefficient of variation of the retention times between the product lots is under 1 % for ***L-column2 ODS*** (Fig. 10). Regardless of the product lot, ***L-column2 ODS*** provides the same results in HPLC analysis because of thorough quality control.



	t_R (1)	t_R (2)	t_R (3)	t_R(4)	t_R (5)	t_R (6)
Lot.A	5.115	5.796	7.008	7.647	9.546	12.50
Lot.B	5.180	5.868	7.103	7.755	9.669	12.64
Lot.C	5.134	5.823	7.022	7.687	9.609	12.60
CV(%)	0.647	0.626	0.725	0.707	0.640	0.575

[Analytical conditions]
Column: ***L-column2 ODS*** (C18, 5μm, 120Å) 4.6×150 mm
Mobile phase: CH_3CN/25 mM Phosphate buffer pH 7.0 (60/40)
Flow rate: 1 mL/min; Temp.: 40 °C; Detection: UV 220 nm; Inj.vol.: 1 μL

Fig. 10 Reproducibility between product lots(basic drugs: antidepressants).

■ Cartification

Specifications and test results of each product lot as well as test results for each column are supplied with the column (Fig. 11). In addtion we support method validation by supplying columns from three different media lots.

[Items of quality assurance]
- Physical properties of the base silica gel:
  - Median particle size (d50)
  - Surface area
  - Median pore diameter
  - Pore volume
  - Metal content
- Properties of the media:
  - Capacity factor of a standard
  - Adsorptive property for basic compounds
  - Adsorptive property for acidic compounds
  - Adsorptive property for chelating compounds
  - Surface hydrophobicity
  - Planar and non-planar compounds separation performance,
  - etc.
- Quality of packing as measured by theoretical plate number

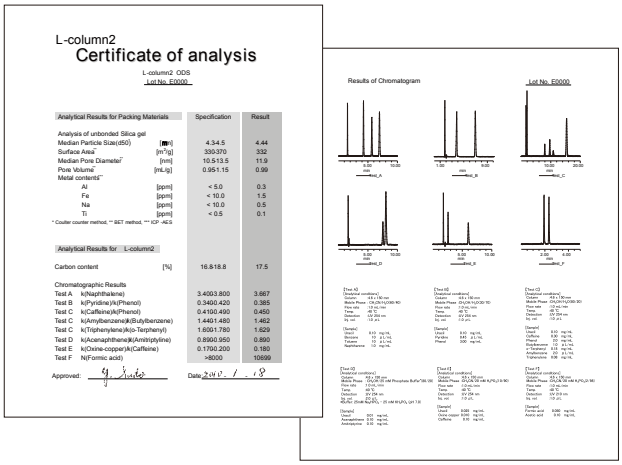


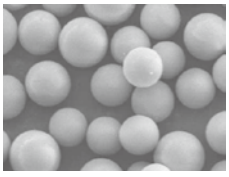
Fig. 11 Certificate at each product lot of packing materials.

■ Base silica gel

High purity silica gel, in which metal impurities are reduced to the absolute limit, is used as the starting material, facilitating analysis of chelating compounds (Table 1).

Table 1 Silica gel test specifications (extract)

Metal impurities	Content (ppm)
Al	≤ 5.0
Fe	≤ 10.0
Ti	≤ 0.5
Mg	≤ 5.0



Specifications silica

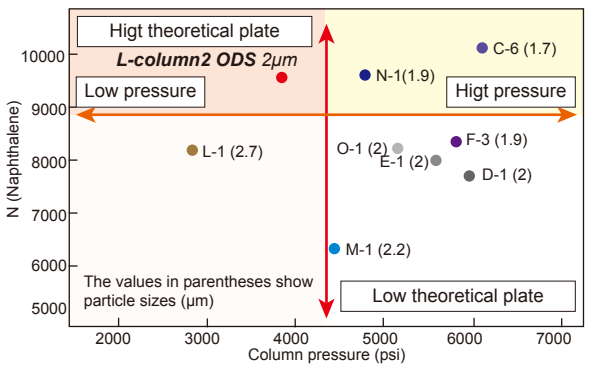
New *L-column2 ODS 2μm*

Innovation of columns for UHPLC

L-column2 ODS 2μm was developed with usability in mind. Its low pressure reduces the load of columns and equipment, and its high durability provides a high theoretical plate number for a wide range of mobile phase flow rates. Therefore, ***L-column2 ODS 2μm*** is an ideal 2-μm particle column.

■ Low back pressure and high theoretical plate number

The back pressure of ***L-column2 ODS*** is low due to thorough quality control and superior packing technology. This means that a high theoretical plate number for a 2-μm particle column can be obtained using a general-purpose HPLC system.
Figure 12 is plot of the theoretical plate number versus back pressure for various brands of columns for UHPLC. The relationship between back pressure and the theoretical plate number of UHPLC columns are clearly different depending on the manufacturer.
L-column2 ODS 2μm is suitable for a wide range of analytical conditions because it has low back pressure in addition to a high theoretical plate number, making it a superior column.

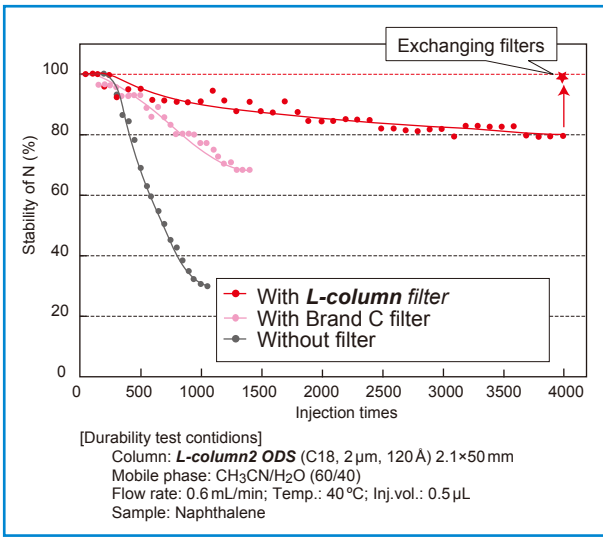


[Analytical conditions]
Column: 2.1×50 mm or 2.0×50 mm (C18)
Mobile phase: CH_3CN/H_2O (50/50)
Flow rate: 0.6 mL/min; Temp.: 25 °C; Detection: UV 254 nm; Inj.vol.: 0.5 μL

Fig. 12 Relationship between column pressure and theoretical plate number.

■ High durability

L-column2 ODS 2μm is packed under very high pressure to endure the high pressures that occur during use. Because it is not influenced by the pressure fluctuations that occur during sample injection, ***L-column2 ODS 2μm*** has the stability for long-term use.
When ***L-column*** *pre-column filter* (page 12 and Fig. 33) is used, the durability of a column is further improved because the filter prevents insoluble elements in the mobile phase and the sample from entering the column (Fig. 13).

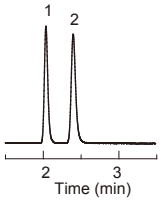
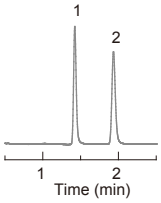
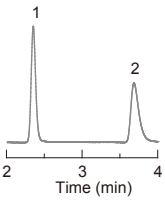
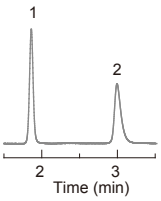
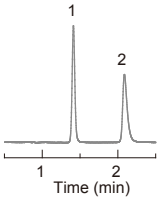
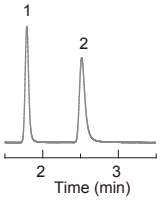
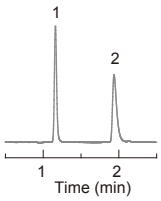


[Durability test contidions]
Column: ***L-column2 ODS*** (C18, 2 μm, 120 Å) 2.1×50 mm
Mobile phase: CH_3CN/H_2O (60/40)
Flow rate: 0.6 mL/min; Temp.: 40 °C; Inj.vol.: 0.5 μL
Sample: Naphthalene

Fig. 13 Effect of pre-column filter on column stability.

■ Low adsorption

Highly accurate ultra-high-speed analyses are possible because there is very low absorption with the advanced end-capping of ***L-column2 ODS 2μm***.
In the analysis of basic amitriptyline and neutral acenaphthene, the tailing coefficient of amitriptyline is close to 1 and the difference in retention times of these two compounds is less when a low-adsorption column is used. ***L-column2 ODS 2μm*** is a superior and low-adsorption column because the tailing coefficient is small, at 1.2, and the difference in retention times of the two compounds is small (Fig. 14).

L-column2 ODS	Brand C-6	Brand D-1	Brand E-1	Brand L-1	Brand M-1	Brand N-1
						
P. 3118 psi	5018 psi	4554 psi	4583 psi	2204 psi	3452 psi	3771 psi
t_R (2) 2.402 min	1.927 min	3.684 min	2.989 min	2.086 min	2.520 min	1.948 min
N (2) 8409	8809	6031	6364	6515	5614	6418
T.F. (2) 1.217	1.222	1.607	1.465	1.539	1.556	1.692

[Analytical conditions]
Column: 2.1×50 mm or 2.0×50 mm (C18)
Moble phase: CH_3CN/25 mM Phosphate buffer pH 7.0 (75/25)
Flow rate: 0.4 mL/min; Temp.: 40 °C; Detection: UV 260 nm; Inj.vol.: 0.5 μL

1. Acenaphthene (I.S.)
2. Amitriptyline

Fig. 14 Comparison between column (basic compound: amytriptyline).

New *L-column2 C8*

Next-generation high-performance silica-based octyl column

Average particle size	5 µm
Average pore size	120 Å
Range of pH	pH 2–7.5
USP category	L7

L-column2 C8 is end-capped using the same advanced method used for ***L-column2 ODS***. This column is easy to use because it provides sharp peaks in addition to it having high durability. ***L-column2 C8*** is most suitable for reducing the analysis time of hydrophobic compounds and conserving solvent.

■ Low adsorption

The residual silanol groups of ***L-column2 C8*** are reduced to the utmost limit by the advanced end-capping method. ***L-column2 C8*** can be used for various compounds, such as acid, basic and chelating compounds.
Figure 15 is a chromatogram of the simultaneous analysis of antihistamines. Although promethazine and clemastine are easily adsorbed by packing materials, ***L-column2 C8*** provides sharp peaks.

■ Reduction of analysis time

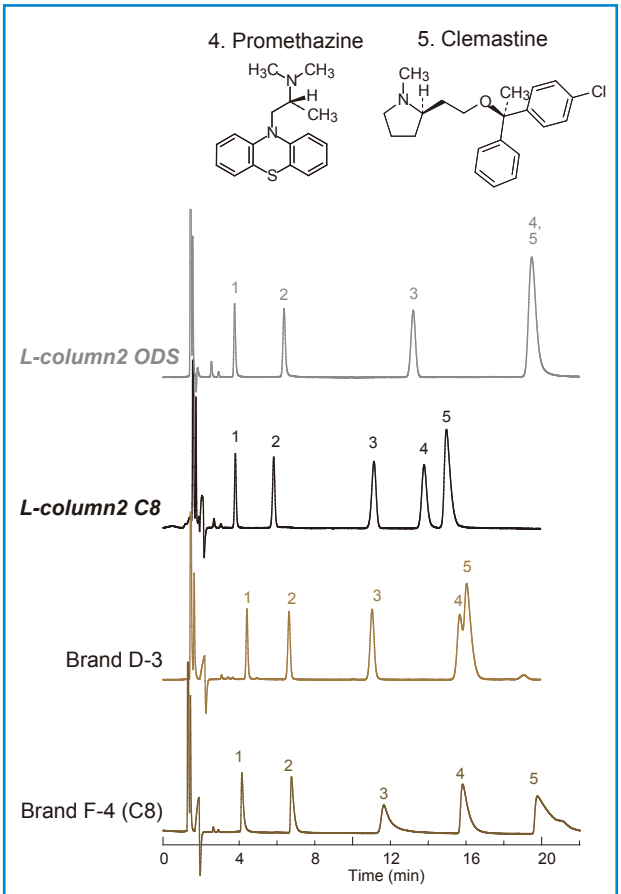
In general, the retention time for C8 columns is short because they have lower retention ability than C18 columns. For more hydrophobic analytes, the analysis time can be shortened, thus reducing the amount of solvent used.
Figure 16 shows results from analysis of lipophilic vitamins using ***L-column2 ODS*** and ***L-column2 C8*** under the same HPLC conditions. Although the analysis of vitamin K using ***L-column2 ODS*** columns takes longer because the lipophilicity of vitamin K is high, the analysis time using ***L-column2 C8*** is one quarter of that using ***L-column2 ODS***.

■ Difference of separation behavior with C18 column

Compared to C18 columns, almost all analytes are eluted faster on C8 columns, but the difference between the retention time for C8 columns and that for C18 columns depends on the analytes. Because the elution behavior of C8 columns is sometimes different from that of C18 columns, C8 columns can sometimes improve separation even if separation using C18 columns is not possible.
Figure 17 shows that ***L-column2 ODS*** cannot thoroughly separate desipramine and paroxetine, while ***L-column2 C8*** can separate them perfectly.

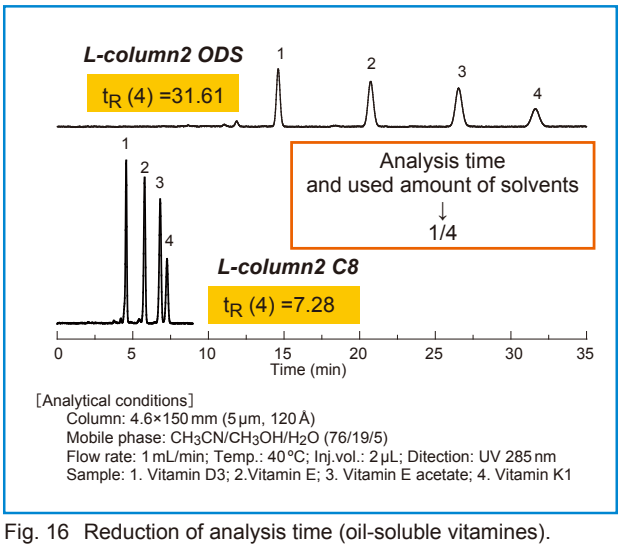
■ High durability

L-column2 C8 is chemically stable and has long-term reliability because it is manufactured using the same advanced end-capping method used for ***L-column2 ODS***.



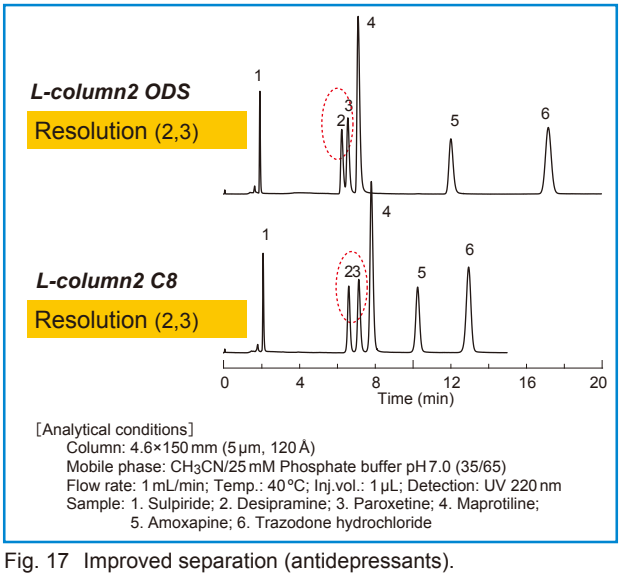
[Analytical conditions]
Column: 2.1×150 mm (5 µm)
Mobile phase: CH_3CN/25 mM Phosphate buffer pH 7.0 (40/60)
Flow rate: 0.2 mL/min; Detection: UV 220 nm; Inj.vol.: 0.5 µL
Sample: 1. Chlorpheniramine; 2.Diphenhydramine;
3. n-Butyl p-hydroxybenzoate; 4. Promethazine; 5. Clemastine

Fig. 15 Chromatogram of antihistamines.



[Analytical conditions]
Column: 4.6×150 mm (5 µm, 120 Å)
Mobile phase: $CH_3CN/CH_3OH/H_2O$ (76/19/5)
Flow rate: 1 mL/min; Temp.: 40 °C; Inj.vol.: 2 µL; Ditection: UV 285 nm
Sample: 1. Vitamin D3; 2.Vitamin E; 3. Vitamin E acetate; 4. Vitamin K1

Fig. 16 Reduction of analysis time (oil-soluble vitamines).



[Analytical conditions]
Column: 4.6×150 mm (5 µm, 120 Å)
Mobile phase: CH_3CN/25 mM Phosphate buffer pH 7.0 (35/65)
Flow rate: 1 mL/min; Temp.: 40 °C; Inj.vol.: 1 µL; Detection: UV 220 nm
Sample: 1. Sulpiride; 2. Desipramine; 3. Paroxetine; 4. Maprotiline;
5. Amoxapine; 6. Trazodone hydrochloride

Fig. 17 Improved separation (antidepressants).

L-column ODS

Established, high-performance standard column

Average particle size	3 µm, 5 µm
Average pore size	120 Å
Range of pH	pH 2–9
USP category	L1

In 1990, ***L-column ODS*** was introduced as a column whose secondary interaction with the residual silanol groups was eliminated by a new end-capping method using high-temperature silylation. At the time, the performance of ***L-column ODS*** was far superior to that of existing C18 columns, and it became a pioneer of the new-generation end-capping method.

■ End-capping method using high-temperature silylation

Various end-capping methods are used by column manufacturers, but the residual silanol groups cannot thoroughly be eliminated by the usual end-capping methods. The residual silanol groups of ***L-column ODS*** are almost entirely eliminated by the high-temperature silylation end-capping method (Fig. 18). Therefore, peak tailing derived from the residual silanol groups does not occur using ***L-column ODS*** and its high durability, even in acidic or basic mobile phases, minimizes deterioration.

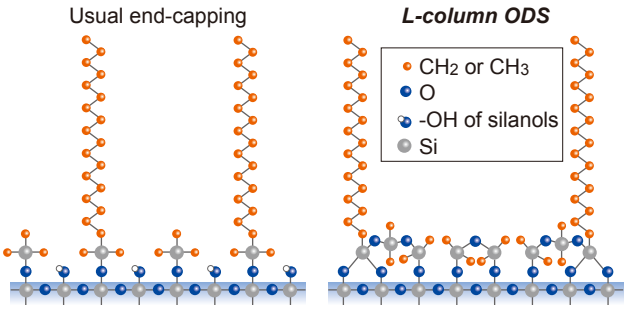


Fig. 18 Veriou design of end-capping.

L-column ODS-P

Wide pore C18 column for analysis of protein and peptide

Average particle size	5 µm
Average pore size	300 Å
Range of pH	pH 2–9
USP category	L1

L-column ODS-P is ideal for the analysis of proteins and peptides. The base silica has a pore diameter of 300 Å. Adsorption is minimized and proteins and peptides elute with sharp peaks. Biological samples are often analyzed using 1 % TFA in the mobile phase and ***L-column ODS-P*** is exceptionally stable in strongly acidic mobile phase.

■ Role of Pore diameter in protein and peptide analysis

Insulin B chain with a molecular weight of 3495 does not show different peak shape between 120 Å pore diameter and 300 Å pore diameter (Fig. 19). Retention is determined by carbon load. On the other hand, myoglobin with a molecular weight of 17400 shows a broad peak when analyzed on the 120 Å ***L-column ODS*** and the main component is not separated from the impurities. Using 300 Å ***L-column ODS-P*** with 300 Å pore diameter, the main component is separated from the impurities with good peak shape. Analytes of molecular weight of approximately 5000 to 20000 are suitable for this column.

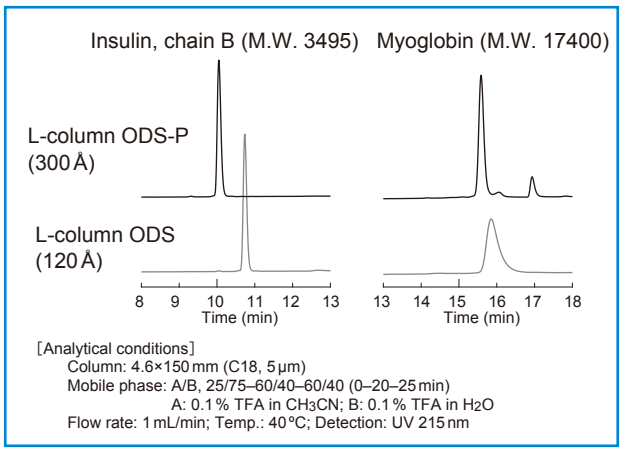


Fig. 19 Peptide and protein analysis using packing materials with different pore diameters.

■ High durability

L-column ODS-P can be used in a pH range from 2 to 9. It demonstrates long lifetimes and stable performance in mobile phases containing 1 % TFA (Fig. 20).

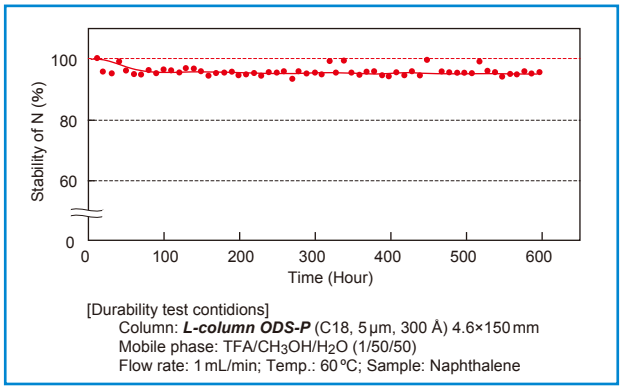


Fig. 20 Durability test with acidic mobile phase.

L-column Micro

High-performance column for nano and micro HPLC

L-column Micro is a nano/micro column with 0.075–0.3 mm I.D. It is a high-performance column that was manufactured by combining low-absorption packing materials, including ***L-column2 ODS***, an originally developed packing technique and a column structure with a small dead volume.

■ Features

- The packing materials can be selected from ***L-column*** series including ***L-column2 ODS***.
- Highly sensitive analyses are possible because of the negligible adsorption of basic peptides and proteins.
- Stabilization time can be shortened and highly sensitive analyses are possible because of low column bleed.
- An inert fused silica capillary is used as the column body, with two kinds of column structures available.

■ Column Structure

[Non-sleeved type]
The chromatography tube is a fused silica capillary. The dead volume of the column is reduced through direct connection of the column with the chromatograph (Fig. 21). This column is most suitable for the identification of phosphorylated peptides by LC/MS/MS because metallic parts are not used.

- Inner diameter: 0.075 mm and 0.1 mm
- External diameter: 0.360 mm

[PEEK-sleeved type]
This column is easy to handle because it is a fused silica capillary with an outer sleeve of PEEK resin. Connection to MS is easy because connecters are attached to the column.

- Inner diameter: 0.075 mm–0.3 mm
- Connection: 1/16 inch stainless-steel connector

■ Proteome analysis

BSA tryptic digest was analyzed using LC/MS/MS and the sample concentration and cover ratio was determined using ***L-column Micro*** and another brand. A higher cover ratio means that more amino acid sequences are read.
L-column Micro shows advantage at all concentration levels, but excels at the lower levels (Fig. 22). With superior end-capping and very high theoretical plate numbers per column, ***L-column Micro*** permits identification of many proteins and is the optimum choice for proteome analysis (Fig. 23).

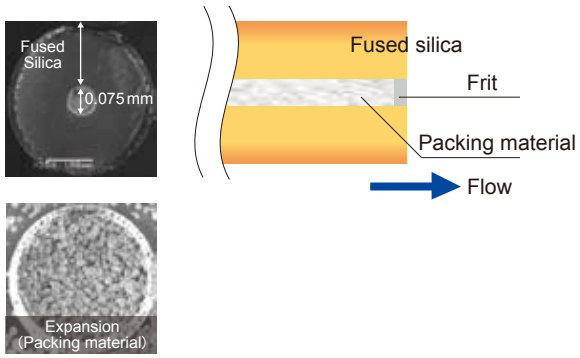
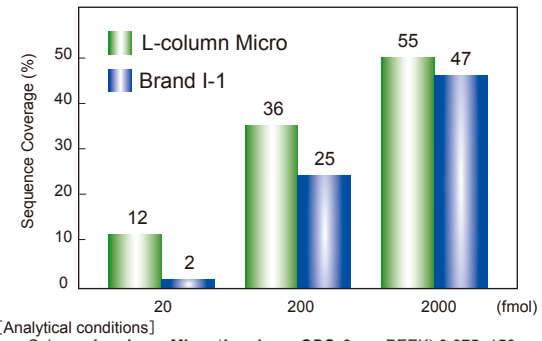
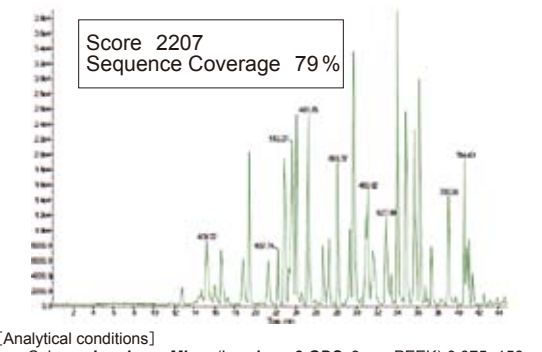


Fig. 21 Electron microgram (non-sleeved type: 0.075 mm I.D.).



[Analytical conditions]
Column: ***L-column Micro*** (***L-column ODS***, 3 µm, PEEK) 0.075×150 mm
Brand I-1 (C18, 3 µm) 0.075×150 mm
Mobile phase: CH_3CN/0.1 % HCOOH in H_2O
Temp.: Ambient
Sample: BSA tryptic digest
System: LC: Cap LC (Waters Co.); MS: Q-TOF micro (Waters Co.)

Fig. 22 Sequence coverage (BSA trypic digest).

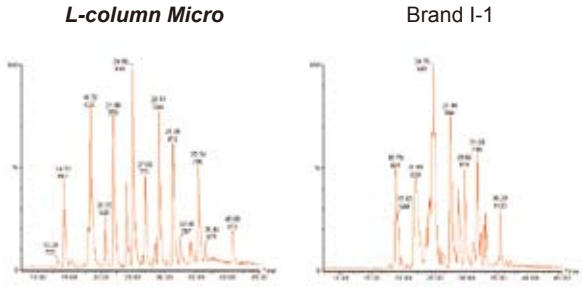


[Analytical conditions]
Column: ***L-column Micro*** (L-***column2 ODS***, 3 µm, PEEK) 0.075×150 mm
Mobile phase: 0.1 % HCOOH in CH_3CN gradient
Flow rate: 0.2 µL/min
Sample: 50 fmol BSA tryptic digest
System: LC: ParadigmMS2+PAL; MS: QSTAR-Elite
※Courtesy of Dr. Ueda, RIKEN center for Genomic Medicine

Fig. 23 Analysis of a small amount of BSA tryptic digest.

■ Low adsorption and high efficiency

L-column Micro can separate many peptides because of the low-adsorption packing materials (Fig. 24). This is effective for the identification of proteins.
The column has a high theoretical plate number and high durability because it is packed homogeneously by a patented technique.



[Analytical conditions]
Column: ***L-column Micro*** (***L-column ODS***, 3 µm, non) 0.075×150 mm
Brand I-1 (C18, 3 µm) 0.075×150 mm
Mobile phase: A/B, 5/95–40/60 (0–30 min)
A: 0.1 % HCOOH in CH_3CN; B: 0.1 % HCOOH in H_2O
Temp.: Ambient; Inj.vol.: 1 µL
Sample: 100 fmol enolase1 tryptic digest
System: LC: Cap LC (Waters Co.); MS: Q-TOF micro (Waters Co.)
Application No. 1073

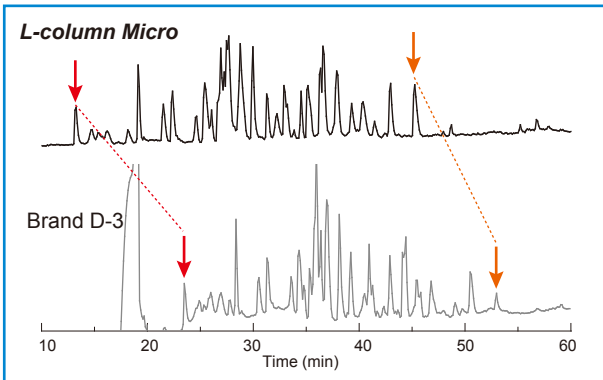
Fig. 24 TIC of enolase1 tryptic digest.

■ Trap column

In proteomic analysis using LC/MS/MS, the trap column is indispensable for increasing the injection volume. A small dead volume, low-adsorption property and high retention ability that traps target constituents are required effectively in a trap column.

[Cartridge trap column]
This trap column does not decrease the theoretical plate number because of its very small dead volume. Not only that, because it is a cartridge-type trap column, it is more economical (Fig. 25).

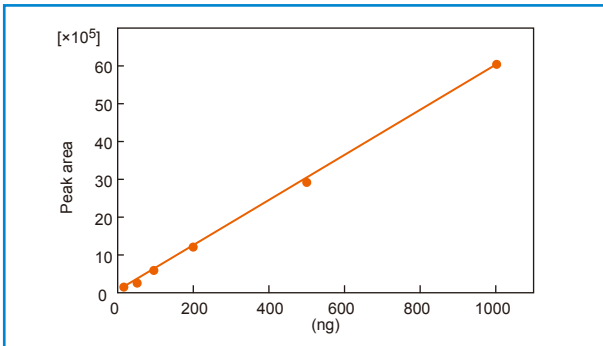
Fig. 25 Structure of Trap column



[Analytical conditions]
Column: ***L-column Micro*** (***L-column ODS***, 3 µm, non) 0.075×150 mm
Trap column: ***L-column Micro*** (***L-column ODS***, 5µm) 0.3×5 mm
Brand D-3 (C18) 0.2×150 mm
Mobile phase: A/B, 5/95–40/60 (0–60 min)
A: 0.1 % HCOOH in CH_3CN; B: 0.1 % HCOOH in H_2O
Detection: UV 215 nm; Inj.vol.: 1 µL
Sample: 500 fmol BSA tryptic digest
Application No. 1071

Fig. 26 Comparison of trap column (BSA tryptic digest).

This trap column retains the target constituents firmly and its media has little irreversible adsorption of the target constituents. Therefore, the actual loss of target constituents is negligible (Fig. 26). In the range of 20–1000 ng for the insulin B chain, a linear relationship is obtained between the injection volume and the peak area (Fig. 27).



[Analytical conditions]
Trap column: ***L-column Micro*** (***L-column ODS***, 5 µm) 0.3×5 mm
Mobile phase: A/B, 5/95–40/60–90/10 (0–10–15 min)
A: 0.1 % HCOOH in CH_3CN; B: 0.1 % HCOOH in H_2O
Detection: UV 215 nm (6 nL cell); Inj.vol.: 1 µL; Sample: Insulin B chaine

Fig. 27 Load capability of trap column (insulin B chain).

Technical date

Selection of media particle size

Although the most popular particle size for reversed phase HPLC remains 5 µm, many manufacturers have introduced 3 µm and sub 2 µm particles into the market for their ability to improve separation and shorten analysis time through use of shorter column length made possible by higher efficiencies and higher optimum flow rates.

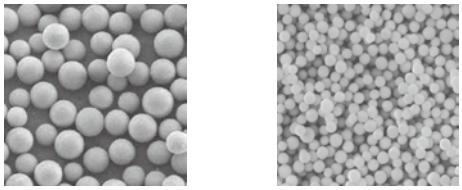


Fig.28 Electron micrograms of silica particles (left: 5 µm; rigth: 2 µm).

Table 2 Relationship of partical size, theoretical plate number and column pressure

Partical size (µm)	Theoretical plate number	Column pressure
2	$\frac{5}{2}$	$\frac{25}{4}$
3	$\frac{5}{3}$	$\frac{25}{9}$
5	1	1
10	$\frac{1}{2}$	$\frac{1}{4}$

■ Particle size and theoretical plate number

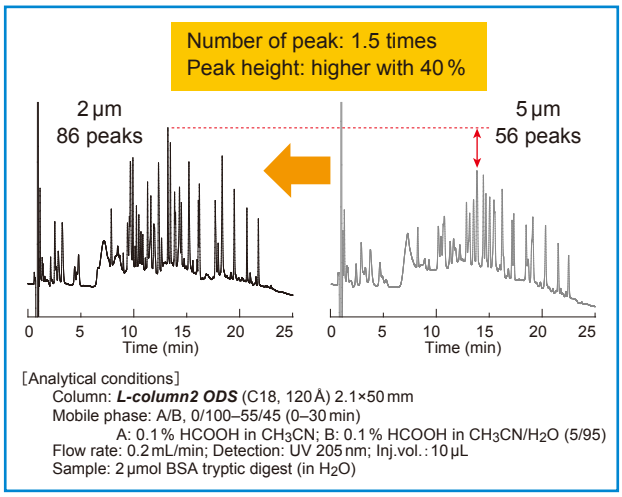
The theoretical plate number is inversely proportional to the particle size (Table 2).
Figure 29 shows the chromatogram of the BSA tryptic digest. Higher and more abundant peaks increase the precision of identifying a protein. In this analysis, 56 peaks were detected using a 5-µm particle column, whereas 86 peaks were detected using a 2-µm particle column, which is 1.5 times more peaks than detected using the 5-µm particle column.

■ Particle size and mobile phase flow rate

The theoretical plate number is influenced by the flow rate of the mobile phase.
Figure 30 shows van Deemter plots for different particle sizes. A low theoretical plate height means high theoretical plate number.
Regarding a 2.1-mm I.D. column, a 5-µm particle column provides a maximum theoretical plate number in the range of 0.2–0.4 mL/min for flow rate, and a 2-µm particle column provides a maximum theoretical plate number in the range of 0.4–0.8 mL/min for flow rate. There is a wide range of optimum flow rates for the 2-µm particle column because the theoretical plate number does not decrease even if the flow rate is increased when using smaller particles. Therefore, a 2-µm particle column is suitable when using high flow rates for the mobile phase.

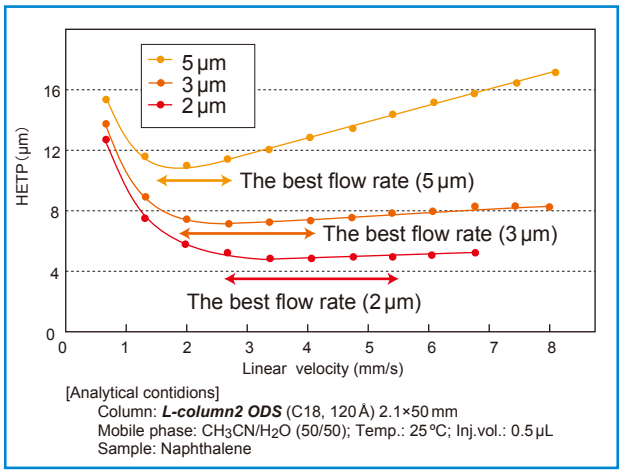
■ Back pressure

The back pressure is high when the particle size is small (Table 2).
Figure 31 shows back pressure versus the organic solvent ratio with respect to particle size under the HPLC conditions in which the column length and the flow rate are optimal. The back pressure is maximal when the ratio of methanol is approximately 50 %.



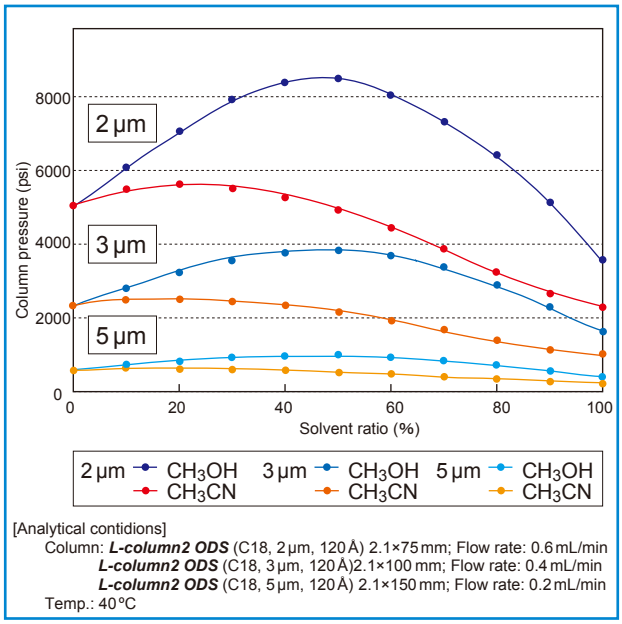
[Analytical conditions]
Column: ***L-column2 ODS*** (C18, 120 Å) 2.1×50 mm
Mobile phase: A/B, 0/100–55/45 (0–30 min)
A: 0.1 % HCOOH in CH_3CN; B: 0.1 % HCOOH in CH_3CN/H_2O (5/95)
Flow rate: 0.2 mL/min; Detection: UV 205 nm; Inj.vol.: 10 µL
Sample: 2 µmol BSA tryptic digest (in H_2O)

Fig. 29 Improvement in separation by changing particle size (BSA tryptic digest).



[Analytical contidions]
Column: ***L-column2 ODS*** (C18, 120 Å) 2.1×50 mm
Mobile phase: CH_3CN/H_2O (50/50); Temp.: 25 °C; Inj.vol.: 0.5 µL
Sample: Naphthalene

Fig. 30 van Deemter plot for each particle size.



[Analytical contidions]
Column: ***L-column2 ODS*** (C18, 2 µm, 120 Å) 2.1×75 mm; Flow rate: 0.6 mL/min
L-column2 ODS (C18, 3 µm, 120 Å)2.1×100 mm; Flow rate: 0.4 mL/min
L-column2 ODS (C18, 5 µm, 120 Å) 2.1×150 mm; Flow rate: 0.2 mL/min
Temp.: 40 °C

Fig. 31 Back pressure versus organic solvent ratio with respect to particle size.

■ Reduction of analytical time (Ultra high-speed analysis)

The analytical time is short when the column is short. Because the theoretical plate number is proportional to column length, the following selections are usually made in order to obtain the same separation capacity as a 5-μm particle column when particle size is smaller than 5 μm and column length is shortened.

5 μm, 250 mm L.→3 μm, 150 mm L.→ 2 μm, 100 mm L.
5 μm, 150 mm L.→ 3 μm, 100 mm L. → 2 μm, 75 mm L.

Figure 32 shows an example of analytical time being shortened by changing particle size and column length. The smaller particle can shorten analytical time because there is less reduction in the theoretical plate number when the flow rate of the mobile phase is increased.

■ Improvement of separation

When the particle size decreases, resolution increases because theoretical plate number increases. The relationship between particle size and resolution is as follows when the resolution of a 5-μm particle column is considered 1.

Resolution of 3 μm particle column is about 1.3 times.
Resolution of 2 μm particle column is about 1.6 times.

A resolution greater than 1.5 is assumed to be almost complete separation. A certain level of improvement in separation can be expected using a smaller particle size when the column size is the same.

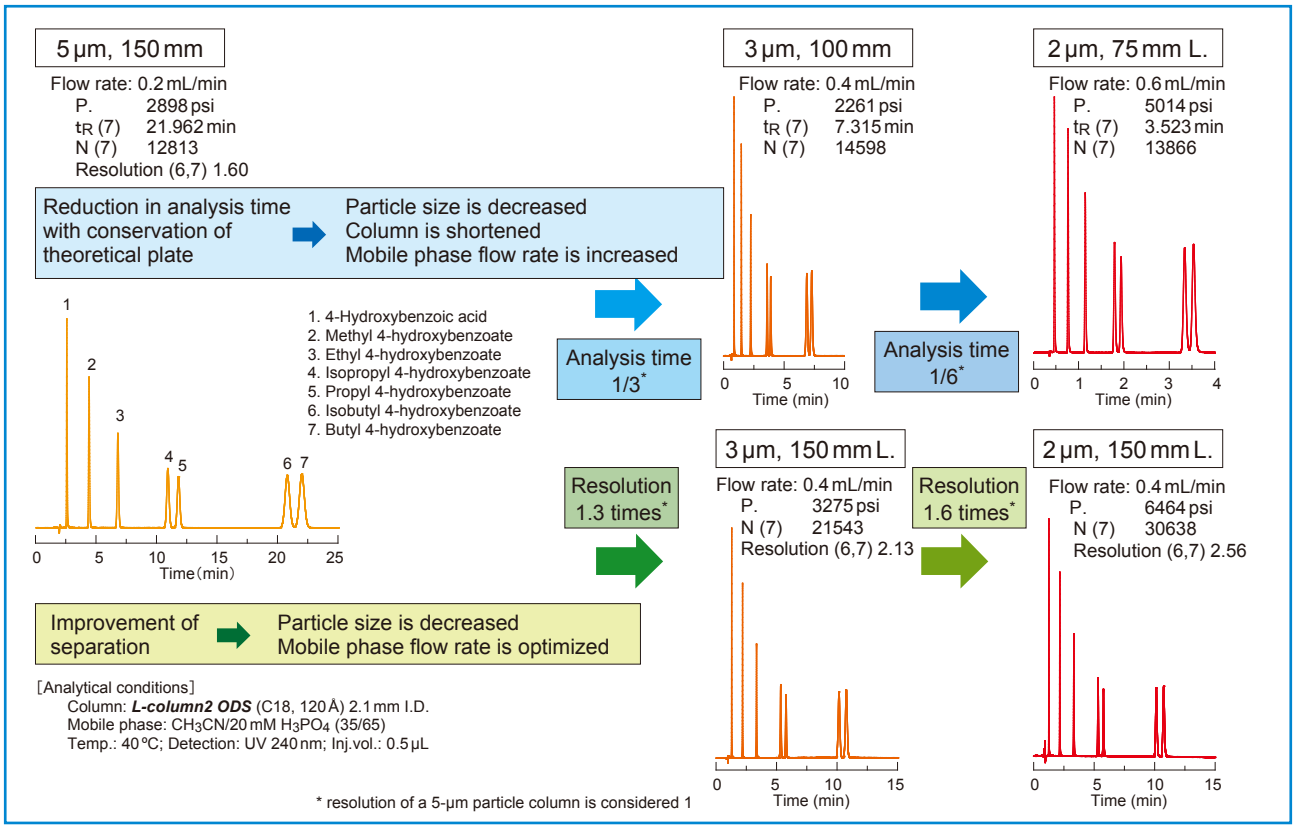


Fig. 32 Reduction in analysis time and improvement of separation by optimization of particle size, column length and mobile phase.

■ Attention when column is used

Because fine particles can easily clog a ≤ 2-μm particle column, mobile phases and samples should be used after filtering with a ≤ 0.45-μm pore-size filter.
The pre-column filter prevents shavings generated from the plunger seal, insoluble matters in samples, etc., from entering the column. The pre-column filter is indispensable for maintaining column longevity.
The theoretical plate number does not decrease when attaching ***L-column*** *pre-column filter* because its direct connection with the analytical column prevents the generation of a dead volume.
Because the types of piping joints may differ between manufacturers, matching the types is necessary to avoid generating a dead volume.
L-column *pre-column filter* can be installed on most HPLC systems because it has two types, Waters and UPLC®.

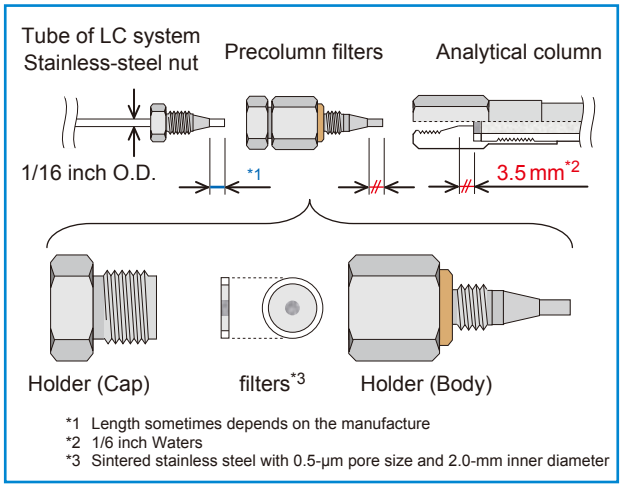


Fig. 33 Structure of ***L-column*** *pre-column filter*.

L-column series lineup

L-column2

L-column2 ODS (patricle size 2 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
2.1	30	713630	
	50	713140	
	75	713640	
	100	713170	
	150	713020	
3.0	30	723650	
	50	723490	
	75	723600	
	100	723330	

L-column2 C8 (Patricle size 5 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
1.5	50	712131	
	100	712161	
	150	712011	
2.1	35	712241	
	50	712141	
	100	712171	
	150	712021	
	250	712221	
4.6	35	722251	
	50	722151	
	100	722181	
	150	722071	
	250	722081	

L-column2 ODS (patricle size 3 µm)

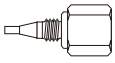
I.D. (mm)	Length (mm)	Cat.No.	Price
1.5	50	711130	
	100	711160	
	150	711010	
2.1	35	711240	
	50	711140	
	100	711170	
	150	711020	
	250	711220	
3.0	50	721490	
	100	721330	
	150	721260	
	250	721320	
4.6	35	721250	
	50	721150	
	75	721460	
	100	721180	
	150	721070	
	250	721080	
20.0	50	741230	

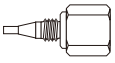
L-column2 ODS (patricle size 5 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
1.5	50	712130	
	100	712160	
	150	712010	
2.1	35	712240	
	50	712140	
	100	712170	
	150	712020	
	250	712220	
3.0	100	722330	
	150	722260	
	250	722320	
4.0	150	722040	
	250	722310	
4.6	35	722250	
	50	722150	
	100	722180	
	150	722070	
	250	722080	
6.0	150	722090	
10.0	150	742510	
	250	742100	
20.0	50	742230	
	150	742520	
	250	742120	

L-column Pre-column filter

	Specification	Cat.No.	Price
First kit [LC connection type: 1/16” Waters (W); column connection type: 1/16” Waters (W)]	1 filter plus holder	653002	
First kit [LC connection type: UPLC®; column connection type: 1/16” Waters (W)]	1 filter plus holder	653004	
Filters	5 filters	653003	

Cat.No.653002  
Connection type: 1/16” Waters 1/16” Waters (W)

Cat.No.653004  
Connection type: 1/16” Waters 1/16” UPLC®

Cat.No.653003 

L-column

L-column ODS (patricle size 3 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
1.5	50	611130	
	100	611160	
	150	611010	
2.1	35	611240	
	50	611140	
	100	611170	
	150	611020	
	250	611220	
3.0	50	621490	
	100	621330	
	150	621260	
	250	621320	
4.6	35	621250	
	50	621150	
	75	621460	
	100	621180	
	150	621070	
	250	621080	
20.0	50	641230	

L-column ODS (patricle size 5 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
1.5	50	612130	
	100	612160	
	150	612010	
2.1	35	612240	
	50	612140	
	100	612170	
	150	612020	
	250	612220	
3.0	100	622330	
	150	622260	
	250	622320	
4.0	150	622040	
	250	622310	
4.6	35	622250	
	50	622150	
	100	622180	
	150	622070	
	250	622080	
6.0	150	622090	
10.0	250	642100	
20.0	50	642230	
	150	642520	
	250	642120	

L-column ODS-P (patricle size 5 µm)

I.D. (mm)	Length (mm)	Cat.No.	Price
2.1	50	612147	
	150	612027	
4.6	50	622157	
	150	622077	

Guard column

L-column2 ODS (patricle size 5 µm)

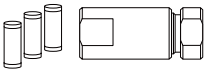
	I.D. (mm)	Length (mm)	Analytical column	Specification	Cat.No.	Price
Cartridge guard column	2.0	5	1.5–3.0 mm I.D.	3 cartridges	752330	
				Set (3 cartridges plus holder)	752331	
	4.6	10	4.0–6.0 mm I.D.	3 cartridges	752050	
				Set (3 cartridges plus holder)	752051	
Guard column	4.0	10	4.0–6.0 mm I.D.		752030	
	10.0	20	10.0–20.0 mm I.D.		752110	

L-column ODS (patricle size 5 µm)

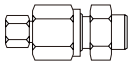
	I.D. (mm)	Length (mm)	Analytical column	Specification	Cat.No.	Price
Cartridge guard column	2.0	5	1.5–3.0 mm I.D.	3 cartridges	652330	
				Set (3 cartridges plus holder)	652331	
	4.6	10	4.0–6.0 mm I.D.	3 cartridges	652050	
				Set (3 cartridges plus holder)	652051	
Guard column	4.0	10	4.0–6.0 mm I.D.		652030	
	10.0	20	10.0–20.0 mm I.D.		652110	

Holder

Specification	Cat.No.	Price
2.0 mm I.D.	652332	
4.6 mm I.D.	652052	



Cartridge guard column



Guard column

L-column Micro

L-column2 ODS (patricle size 3 µm)

I.D. (mm)	Length (mm)	Type	Cat.No.	Price
0.075	50	Non-sleeved	711370	
		PEEK-sleeved	711410	
	150	Non-sleeved	711380	
		PEEK-sleeved	711420	
0.1	50	Non-sleeved	711350	
		PEEK-sleeved	711390	
	150	Non-sleeved	711360	
		PEEK-sleeved	711400	
0.2	50	PEEK-sleeved	711290	
	150	PEEK-sleeved	711300	
0.3	50	PEEK-sleeved	711270	
	150	PEEK-sleeved	711280	

L-column2 ODS (patricle size 5 µm)

I.D. (mm)	Length (mm)	Type	Cat.No.	Price
0.075	50	Non-sleeved	712370	
		PEEK-sleeved	712410	
	150	Non-sleeved	712380	
		PEEK-sleeved	712420	
0.1	50	Non-sleeved	712350	
		PEEK-sleeved	712390	
	150	Non-sleeved	712360	
		PEEK-sleeved	712400	
0.2	50	PEEK-sleeved	712290	
	150	PEEK-sleeved	712300	
0.3	50	PEEK-sleeved	712270	
	150	PEEK-sleeved	712280	

L-column ODS (patricle size 3 µm)

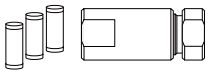
I.D. (mm)	Length (mm)	Type	Cat.No.	Price
0.075	50	Non-sleeved	611370	
		PEEK-sleeved	611410	
	150	Non-sleeved	611380	
		PEEK-sleeved	611420	
0.1	50	Non-sleeved	611350	
		PEEK-sleeved	611390	
	150	Non-sleeved	611360	
		PEEK-sleeved	611400	
0.2	50	PEEK-sleeved	611290	
	150	PEEK-sleeved	611300	
0.3	50	PEEK-sleeved	611270	
	150	PEEK-sleeved	611280	

L-column ODS (patricle size 5 µm)

I.D. (mm)	Length (mm)	Type	Cat.No.	Price
0.075	50	Non-sleeved	612370	
		PEEK-sleeved	612410	
	150	Non-sleeved	612380	
		PEEK-sleeved	612420	
0.1	50	Non-sleeved	612350	
		PEEK-sleeved	612390	
	150	Non-sleeved	612360	
		PEEK-sleeved	612400	
0.2	50	PEEK-sleeved	612290	
	150	PEEK-sleeved	612300	
0.3	50	PEEK-sleeved	612270	
	150	PEEK-sleeved	612280	

Cartridge trap column (patricle size 5 µm)

I.D. (mm)	Length (mm)	Bulking agent's kind	Specificatio	Cat.No.	Price
0.3	5	***L-column2 ODS***	3 cartridges	752450	
		L-column ODS	3 cartridges	652450	
–	–	–	Holder	652452	



Cartridge trap column

- Connection type: 1/16 inch Waters (W)
- A tube and a connector, used to connect a guard or trap column to an analytical column, are not attached.
- Please contact CERI for dimensions not listed here. Columns with a wide range of inner diameters are available, from the nano column with a 0.075-mm inner diameter to the preparative column with a 50-mm inner diameter.

Chemicals Evaluation and Research Institute, Japan

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ISO 9001

Design,Development,Production
and supply of Column
for GC and HPLC